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Lactococcin G Is a Potassium Ion-Conducting, Two-Component Bacteriocin

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Lactococcin G is a novel lactococcal bacteriocin whose activity depends on the complementary action of two peptides, termed α and β. Peptide synthesis of the α and β peptides yielded biologically active lactococcin G, which was used in mode-of-action studies on sensitive cells of *Lactococcus lactis*. Approximately equivalent amounts of both peptides were required for optimal bactericidal effect. No effect was observed with either the α or β peptide in the absence of the complementary peptide. The combination of α and β peptides (lactococcin G) dissipates the membrane potential (Δψ), and as a consequence cells release α-aminoisobutyrate, a non-metabolizable alanine analog that is accumulated through a proton motive-force dependent mechanism. In addition, the cellular ATP level is dramatically reduced, which results in a drastic decrease of the ATP-driven glutamate uptake. Lactococcin G does not form a proton-conducting pore, as it has no effect on the trans-membrane pH gradient. Dissipation of the membrane potential by uncouplers causes a slow release of potassium (rubidium) ions. However, rapid release of potassium was observed in the presence of lactococcin G. These data suggest that the bactericidal effect of lactococcin G is due to the formation of potassium-selective channels by the α and β peptides in the target bacterial membrane.

Bacteriocins produced by lactic acid bacteria are peptides displaying bactericidal activity against gram-positive bacteria, particularly closely related species. The study of such antibacterial agents is of interest because of their potential application as food additives. Most bacteriocins produced by lactic acid bacteria are small peptides with sizes of 35 to 60 amino acid residues. The antimicrobial activities of most bacteriocins studied so far require the action of a single peptide, which is thought to form nonselective pores according to the "barrel stave" mechanism (19). Bacteriocin activity of lactococcin G is associated with the complementary action of two peptides termed α and β (17). The α and β peptides have molecular masses of 4,346 and 4,110 Da, consist of 39 and 35 amino acids, and have isoelectric points of 10.9 and 10.4, respectively. The amino-terminal halves of both peptides may form amphiphilic α helices and may oligomerize in such a way that the nonpolar side of the amphiphilic α-helix region faces the membrane lipids, while the polar side faces the center of the pore, as described for the barrel stave mechanism.

Peptide synthesis yielded biologically active lactococcin G which was used to study the impact of lactococcin G on the energy-transducing properties of sensitive cells of *Lactococcus lactis*. Our data suggest that lactococcin G has a novel bactericidal activity in forming potassium-selective channels in the target cells rather than nonselective pores.

MATERIALS AND METHODS

Bacteriocin assay. Bacteriocin activity was measured as previously described (17), using a microtiter plate assay system. Briefly, 200 μl of culture medium (supplemented with 0.1% [vol/vol] Tween 80), bacteriocin fractions at twofold dilutions, and the indicator strain (A_{600} of 0.1) were added to each well of a microtiter plate. The microtiter plate cultures were then incubated for 3 to 5 h at 30°C, after which growth inhibition of the indicator strain was measured spectrophotometrically at 600 nm by use of a microplate reader. One bacteriocin unit was defined as the amount of bacteriocin that inhibited the growth of the indicator strain by 50% (50% of the turbidity of the control culture without bacteriocin). Specific activity of bacteriocin fractions was defined as the number of bacteriocin units per milliliter in the fraction divided by the optical density at 280 nm of the fraction.

Purification of natural lactococcin G, and peptide synthesis, and purification and analysis of the polypeptides. Natural lactococcin G α and β peptides were purified to homogeneity from 1- to 2-liter cultures of *L. lactis* LMG 2081 by ammonium sulfate precipitation and cation exchange, hydrophobic interaction, and reverse-phase chromatography, all as previously described (17).

The α and β peptides were synthesized according to the amino acid sequence reported earlier (17). This sequence has subsequently been confirmed by sequencing the gene encoding lactococcin G (16). For purification, the synthesized peptides were solubilized in 0.1% trifluoroacetic acid (TFA) and applied to a C_2/C_{18} reverse-phase column (PepRPC HR 5/5) equilibrated with 0.1% TFA. The peptide fragments were eluted with a linear gradient ranging from 30 to 40% 2-propanol containing 0.1% TFA. The fraction containing the most bacteriocin activity was diluted four- to fivefold with H_2O containing 0.1% TFA and rechromatographed on the reverse-phase column. This was repeated two to three times, until homogeneous fractions of the synthetic lactococcin G α and β peptides were obtained. The primary structures and purity of the peptides were confirmed by protein sequencing (Applied Biosystems [Foster City, Calif.] automatic sequencer with on-line 120A phenylthiohydantoin amino acid analyzer), mass spectroscopy analysis on a Sciex (Thornhill, Ontario, Canada) API III LC/MS/MS system, and capillary electrophoresis (Beckman P/ACE System 2050).

Strains and culture conditions. The bacteriocin producer *L. lactis* LMG 2081, which was also used in control experiments as a nonsensitive strain, was obtained from J. Narvhus, Agricultural University, Ås, Norway. *L. lactis* IL1403 (5) was used as a sensitive strain. Both strains were grown at 30°C in M17 broth (Oxoid) without lactose but supplemented with 0.5% (wt/vol) glucose and with or without potassium-L-malate (50 mM) and harvested in the logaritmic growth phase.

Transport assays. Cells were harvested by centrifugation, washed, and resuspended in 50 mM potassium phosphate (pH 7.0) supplemented with 10 mM $MgSO_4$. For transport experiments, the concentrated cell suspension was diluted to a final protein concentration of 0.5 to 1.0 mg/ml into 50 mM potassium phosphate (pH 7.0) containing 10 mM glucose. After 2 min of preenergization at 30°C, radiolabelled solutes were added, and after the indicated periods, the uptake reaction was stopped by the addition of 2 ml of ice-cold 0.1 M LiCl. Samples were filtered over 0.45-μm-pore-size cellulose nitrate filters (Millipore

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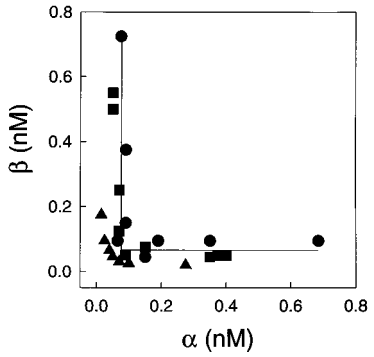

FIG. 1. Synthetic and natural lactococcin G have similar bactericidal activities, and optimal activity occurs at a 1:1 molar ratio of the α and β peptides. Bactericidal activities of natural (▲) and synthetic (two experiments [●, ■]) α and β peptides on *L. lactis* IL1403 were assayed with the microwell assay as described previously (17).

Corp.) and washed once more with 2 ml of ice-cold 0.1 M LiCl as described previously (8).

Measurements of proton motive force. The membrane potential ($\Delta\psi$) was measured by using the fluorescent probe 3,3′-dipropylthiadicarbocyanine iodide [$diSC_3(5)$]. Second, the distribution of tetraphenylphosphonium ion (TPP^+) was measured with a TPP^+-selective electrode (12). The magnitude of the $\Delta\psi$ was calculated according to the Nernst equation. A correction for TPP^+ binding to the cells according to Lolkema et al. (13) was made. For determination of bound TPP^+, cells were deenergized by treatment with 1% (vol/vol) toluene for 30 min at 30°C.

The transmembrane pH gradient (ΔpH) was measured by loading cells with the fluorescent pH indicator 2′,7′-bis-(2-carboxyethyl)-5(and-6)-carboxyfluorescein (BCECF) as described previously (15). Alternatively, ΔpH was measured by monitoring the distribution of radiolabelled benzoic acid by the silicon oil centrifugation technique (3, 11).

Miscellaneous methods. Protein content and cellular ATP levels were determined as described previously (14, 20). ATP depletion was obtained after 30 min of incubation with 20 mM freshly prepared deoxyglucose at 30°C. Radioactivity was measured by liquid scintillation counting in a Packard Tri-Carb 460 CD counter (Packard Instruments Corp.).

Materials. Rubidium-86 (1 mCi/1.1 mg), amino-2-[1-C]isobutyrate (59 mCi/mmol), L-[U-^{14}C]leucine (310 mCi/mmol), L-[U-^{14}C]glutamate (270 mCi/mmol), and [*carboxyl*-^{14}C]benzoic acid (22 mCi/mmol) were obtained from Amersham UK. The peptides were dissolved in 55% (vol/vol) isopropanol and 0.1% (vol/vol) TFA and mixed in a 1:1 ratio. Controls received identical amounts of the solvent. All other chemicals were reagent grade and obtained from commercial sources.

RESULTS

Peptide synthesis of the lactococcin G α and β peptides yields biologically active bacteriocin: relative amounts of α and β necessary to obtain bacteriocin activity. Synthetic and natural lactococcin G had comparable bactericidal activities, as shown in Fig. 1, where the amounts of the α and β peptides (both natural and synthetic), which in combination inhibited growth of the indicator organism by 50%, are plotted as an isohologram. When α was present in excess (more than 0.1 nM), the presence of 0.05 to 0.1 nM β per well resulted in 50% growth inhibition (Fig. 1). Similarly, when β was in excess (more than 0.1 nM), the presence of 0.05 to 0.1 nM α resulted in 50% growth inhibition (Fig. 1). When neither α nor β was in excess, 0.05 to 0.1 nM each also resulted in 50% growth inhibition. Thus, equivalent amounts of both peptides were required. Neither α nor β at concentrations as high as 50 μM had activity in the absence of the complementary peptide. Peptide synthesis thus proved to be a simple method by which biologically active α and β lactococcin G peptides may be obtained in amounts and with purity which allow structural and mode-of-action studies. It should be noted that the concentrations used in the experiments of Fig. 1 are much lower than those used in the experiments of Fig. 2 to 7 because of the length of the incubation, 3 to 5 h, and the presence of 0.1% Tween 80.

Lactococcin G inhibits amino acid uptake. To reveal its primary mechanism of action, the effect of lactococcin G on amino acid transport by the sensitive strain *L. lactis* IL1403 was studied. Uptake of alanine and its nonmetabolizable analog α-aminoisobutyric acid (AIB) and of L-leucine by *L. lactis* cells occurs in cotransport with a proton(s) and is driven by the proton motive force (6–8). Glucose-energized cells rapidly accumulated the AIB (Fig. 2) and leucine. In the presence of the α and β peptides (1:1) of lactococcin G, uptake of AIB was completely inhibited (Fig. 2). AIB accumulated by the cells was rapidly released upon the addition of the α and β peptides (1:1) of lactococcin G. Similar effects of lactococcin G on leucine transport were observed (data not shown). The α and β peptides of lactococcin G alone at 29 nM had no effect on AIB or leucine transport or on accumulated AIB and leucine (data not shown). In contrast to the sensitive strain, lactococcin G did not inhibit AIB or leucine transport in the producer strain *L. lactis* LMG 2081 (data not shown). To analyze the cause of the inhibition of AIB and leucine transport, the effect of lactococcin G was determined in cells energized with an alternative energy source, malate. Under those conditions, proton motive force generation is the direct result of carrier-mediated electrogenic exchange between malate and lactate and the decarboxylation of malate by the malic enzyme (22). The effects of lactococcin G on AIB and leucine transport were identical when malate was used as an energy source instead of glucose (data not shown). These observations indicate that neither the glycolytic pathway nor malate decarboxylation is the primary target of lactococcin G.

Glutamate uptake by *L. lactis* is, unlike the alanine and leucine transport systems, dependent on ATP rather than the proton motive force, and transport appears to be unidirectional (23). Uptake of glutamate was blocked shortly after addition of lactococcin G (35 nM), but no release of glutamate occurred (Fig. 3). Addition of an excess of lactococcin G (35

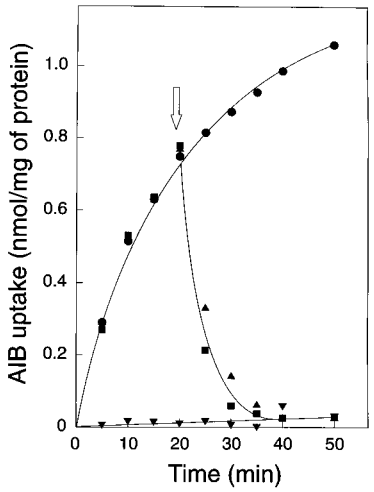

FIG. 2. Effect of lactococcin G on the uptake of AIB by glucose-energized cells of *L. lactis* IL1403. Washed cells (0.5 mg of protein per ml) were suspended in 50 mM potassium phosphate (pH 7.0)–10 mM $MgSO_4$–0.5% (wt/vol) glucose; after 2 min of preincubation, 4.2 μM [^{14}C]AIB (59 mCi/mmol) was added and uptake was monitored. At the arrow, either 3.35 μM α peptide and 3.35 μM β peptide of lactococcin G (■) or 5 μM nigericin and 5 μM valinomycin (▲) were added or solvent was added (●). The effects of lactococcin G were also observed at concentrations as low as 1.2 nM (data not shown). In a parallel experiment, the α and β peptides of lactococcin G were added prior to the addition of AIB (▼).

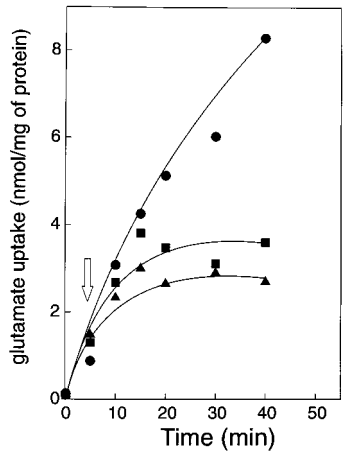

FIG. 3. Effect of lactococcin G on the uptake of glutamate by glucose-energized cells of *L. lactis* IL1403. Transport of L-[^{14}C]glutamate was measured as described in Materials and Methods. At time zero (▲) or at 5 min (■), 35 nM α peptide and 35 nM β peptide of lactococcin G were added or solvent was added (●). Addition of only the α peptide or only the β peptide gave results similar to those for the control (●).

μM) also did not cause efflux of glutamate (data not shown). This result suggests that the inhibition of glutamate uptake by lactococcin G is not caused by the formation of pores that are large enough to release glutamate. High-pressure liquid chromatography analysis of the intracellular amino acid pools indicated that in the presence of lactococcin G, most amino acids are retained by the cell (data not shown). In contrast, nisin, which forms large specific pores in the membrane (9), caused a complete loss of amino acids (data not shown).

Lactococcin G lowers the cellular ATP level. Since transport of glutamate is an ATP-requiring process, the impact of lactococcin G on the cellular ATP level was investigated. Upon addition of glucose to *L. lactis*, the intracellular ATP concentration increased more than fourfold (Fig. 4). Upon the addition of lactococcin G, the cellular ATP level slowly dropped to

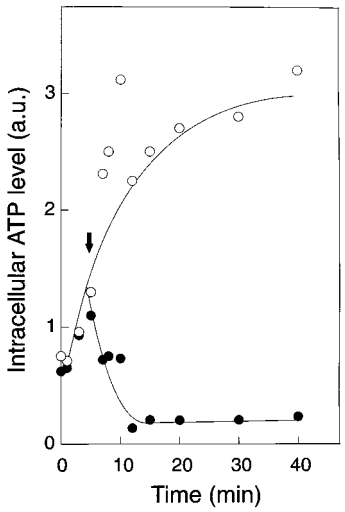

FIG. 4. Effect of lactococcin G on the intracellular ATP level in *L. lactis* IL1403 cells. ATP measurements were performed as indicated in Materials and Methods. a.u., arbitrary units. Glucose was added at time zero. At the arrow, either 35 nM α peptide and 35 nM β peptide of lactococcin G were added (●) or solvent was added (○).

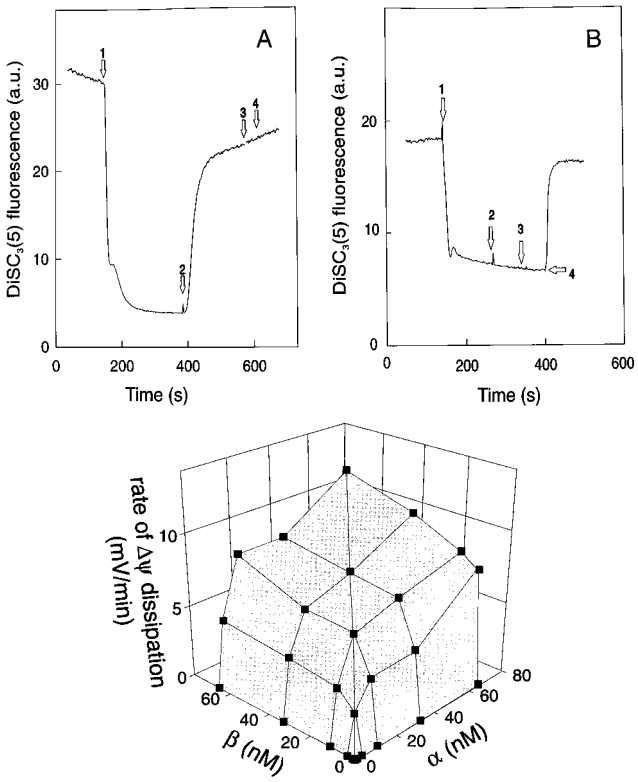

FIG. 5. Effects of lactococcin G on the Δψ in *L. lactis* IL1403 (A and C) and *L. lactis* LMG 2081 (B) cells. The Δψ was measured by using 3 μM the fluorescent probe diSC$_3$(5) and excitation and emission wavelengths of 643 and 666 nm, respectively. Cells (18 μg of protein per ml) were suspended in 50 mM potassium phosphate (pH 7.0) and energized by 0.5% (wt/vol) glucose (A and B, arrows 1). Subsequently 35 nM α peptide and 35 nM β peptide of lactococcin G were added (A, arrow 2; B, arrows 2 and 3). Addition of 0.5 μM valinomycin to dissipate Δψ completely: A, arrows 3 and 4; B, arrow 4. a.u., arbitrary units. (C) Dissipation rate of Δψ by combinations of α and β peptides. Cells were added to TPP$^+$-containing buffer and energized with 0.5% glucose. In the presence of 1 μM nigericin, α and β peptides were added, and the rate of Δψ dissipation was recorded with a TPP$^+$-selective electrode.

levels which are significantly lower than those before the addition of lactococcin G (Fig. 4). An increase in the extracellular ATP level was not observed (data not shown).

Lactococcin G selectively dissipates the Δψ component of the proton motive force. Inhibition of AIB, leucine, and glutamate transport by lactococcin G could directly or indirectly be caused by an effect of lactococcin G on the proton motive force. Therefore, the effect of lactococcin G on Δψ and ΔpH was determined in the sensitive strain *L. lactis* IL1403 and the insensitive strain LMG 2081. A fluorometric assay using the cyanine dye diSC$_3$(5) was applied to monitor Δψ. When cells were supplied with glucose, a rapid quenching of the fluorescence was observed, indicating the generation of a Δψ, inside negative (Fig. 5A and B). Subsequent addition of lactococcin G resulted in a complete collapse of the Δψ in the sensitive strain (Fig. 5A). In contrast, lactococcin G had no effect on the Δψ in the producer strain, *L. lactis* LMG 2081 (Fig. 5B). When the α and β peptides were tested separately in the sensitive strain, no collapse of the Δψ was observed (data not shown). Similar results were obtained when Δψ was monitored with a TPP$^+$-selective electrode. The α and β peptides were added in a range of ratios to energized sensitive cells, and the rate of Δψ dissipation was measured with the TPP$^+$ electrode. Figure 5C shows the effects of different combinations of α- and β-peptide

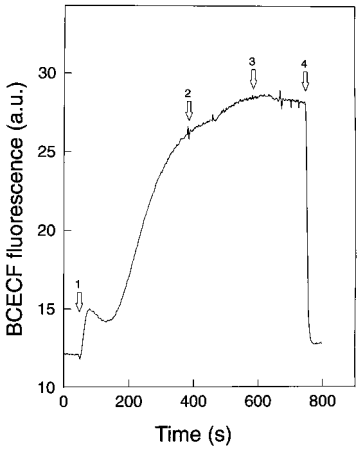

FIG. 6. Lactococcin G does not dissipate the ΔpH. Cells were loaded with BCECF as described in Materials and Methods. BCECF fluorescence measurements were performed by using excitation and emission wavelengths of 502 and 525 nm with slit widths of 5 and 15 nm, respectively. Cells (10 μg of protein per ml) were suspended in 50 mM potassium phosphate (pH 7.0) and energized by 0.5% (wt/vol) glucose. Glucose was added at arrow 1. At arrow 2, 0.5 μM valinomycin was added to dissipate the Δψ completely and to maximize the ΔpH. Next (arrow 3), 29 nM α peptide and 29 nM β peptide of lactococcin G were added, followed by the addition of 1 μM nigericin (arrow 4) to collapse the ΔpH. a.u., arbitrary units.

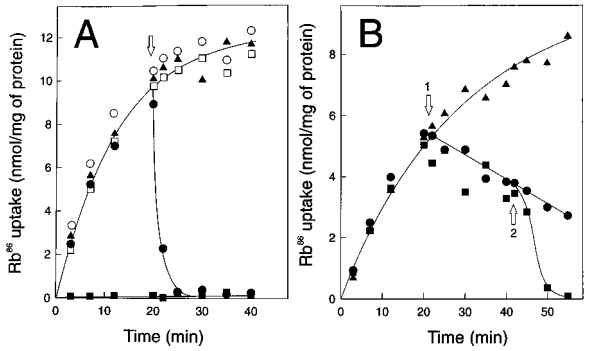

FIG. 7. (A) Lactococcin G causes the efflux of intracellular rubidium ions. Transport of $^{86}Rb^+$ was measured as described in Materials and Methods. Cells were washed and suspended in 50 mM sodium phosphate (pH 7.0)–10 mM $MgSO_4$ and energized with 0.5% (wt/vol) glucose. Uptake was initiated by the addition of ^{86}RbCl (0.55 μg, 0.5 μCi), and at the arrow, 29 nM α peptide and 29 nM β peptide of lactococcin G (●), 29 nM α peptide alone (□), 29 nM β peptide alone (○), or solvent (▲) was added. In another experiment, 29 nM α peptide and 29 nM β peptide were added prior to the addition of ^{86}RbCl (■). (B) Lactococcin G is more effective than uncouplers to induce the release of rubidium ions from *L. lactis* IL1403 cells. Uptake was initiated by the addition of ^{86}RbCl (0.55 μg, 0.5 μCi), and at arrow 1, 50 μM the uncoupler CCCP (●, ■) or 0.5% (vol/vol) ethanol (▲) was added. At arrow 2, 29 nM α peptide and 29 nM β peptide of lactococcin G were added (■) or solvent was added (●).

concentrations on the Δψ dissipation rate. Neither the α peptide alone nor the β peptide alone caused dissipation of the Δψ. Dissipation of the Δψ started when both α and β peptides simultaneously had concentrations of between 1 and 3 nM, that is, between 0.6 and 1.8 pmol/mg of cell protein. Detectable Δψ-dissipating activity of lactococcin G always led to complete Δψ dissipation.

Next, we investigated the effect of lactococcin G on the ΔpH component of the proton motive force. Two techniques were used for this purpose: (i) the fluorescence of the probe BCECF loaded into the cells and (ii) the distribution of radiolabelled benzoic acid. The BCECF fluorescence level rapidly increased after the addition of glucose, indicating an increase of the internal pH (Fig. 6). Addition of lactococcin G had no effect on the BCECF fluorescence level, whereas the signal was rapidly reversed upon the addition of the ionophore nigericin. Similar results were obtained when the distribution of the weak acid benzoate was measured (Table 1). Both techniques clearly demonstrate that lactococcin G does not affect the ΔpH. These data suggest that lactococcin G selectively dissipates the Δψ and that this dissipation is not due to an increased proton permeability of the membrane.

Lactococcin G elicits rubidium efflux. Dissipation of the Δψ by lactococcin G without affecting the ΔpH implies that the membranes become permeable to ions other than protons. The most abundant ion accumulated by lactococcal cells is potassium, which can reach intracellular levels of up to 800 mM (21). To analyze the impact of lactococcin G on the intracellular potassium pool, rubidium-86 was used as a tracer. In the absence of lactococcin G as well as in the presence of either the α peptide alone or the β peptide alone, cells rapidly accumulated the rubidium, and the subsequent addition of lactococcin G caused immediate efflux of rubidium (Fig. 7A). Lactococcin G, present before the addition of rubidium, completely inhibited rubidium uptake by *L. lactis* IL1403 (Fig. 7A). In contrast to the addition of lactococcin G, dissipation of the proton motive force by the uncoupler 50 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) resulted only in a slow release of the rubidium (Fig. 7B). Subsequent addition of lactococcin G again resulted in an immediate and complete release of the rubidium (Fig. 7B). If CCCP was added prior to rubidium, the rubidium uptake was not completely blocked but was around six times lower than in the absence of CCCP (data not shown). If following rubidium uptake cells were ATP depleted, rubidium leaked slowly out of the cells during the ATP depletion. Subsequent addition of lactococcin G caused a rapid efflux of the remaining rubidium (data not shown). These data suggest that lactococcin G dissipates the Δψ by making the membrane permeable for potassium ions.

TABLE 1. Effect of lactococcin G on the steady-state ΔpH

Addition	ΔpH (U)[a]
Glucose	0.79 ± 0.07
Glucose + lactococcin G[b]	0.81 ± 0.04
Glucose + valinomycin-nigericin[c]	0.05 ± 0.004

[a] Measured by the distribution of [^{14}C]benzoic acid present at 16 μM.
[b] 5.9 nM α peptide and 5.9 nM β peptide.
[c] 1 μM each.

DISCUSSION

Peptide synthesis followed by purification by reverse-phase chromatography proved to be a relatively simple procedure by which biologically active lactococcin G may be obtained in amounts and with purity sufficient for structural and mode-of-action studies. The fact that lactococcin G produced by peptide synthesis was biologically active indicates that the previously determined primary structures for this bacteriocin are sufficient to induce activity. This is not a trivial point, as lactococcin G may have had unidentified modifications required for activity. For instance, D-alanine was recently identified in the LAB-bacteriocin lactocin S (24), and this type of modification is not readily detected upon amino acid sequencing.

One cannot be certain that the α and β peptides are purified such that they are completely free from each other during isolation of natural lactococcin G. Upon peptide synthesis,

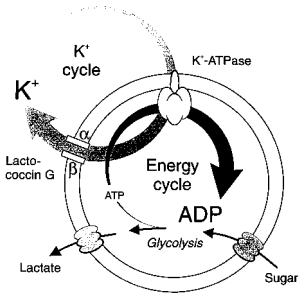

FIG. 8. Model of lactococcin G-mediated cell killing.

however, the α and β peptides are obtained completely free of each other. Thus, peptide synthesis of the α and β peptides allowed us to investigate to what extent each peptide alone exerts bactericidal activity. Neither synthetic α nor synthetic β peptide showed bactericidal activity in the absence of the complementary peptide, indicating that the low, though significant, activity detected for the natural peptides, when each peptide is assayed alone, may be due to the presence of low-level contamination of the complementary peptide.

In an earlier study, we estimated that approximately 0.05 nM β peptide and 0.3 nM α peptide were required to obtain 50% growth inhibition (17). The somewhat higher amounts of the α peptide needed in the earlier study (0.3 nM, compared with 0.1 to 0.05 nM) suggested that an excess of α over β peptide was needed. However, the present data indicate that the two peptides interact in approximately equivalent amounts. The somewhat lower specific activity for the α peptide originally observed may have been due to inactivation (natural α peptide may exist in a form with reduced bacteriocin activity [17]) and/or contaminations that may have been present in the α-peptide fractions which were analyzed. An interaction between the α and β peptides in an approximately 1:1 ratio is consistent with the fact that their genes are expressed from the same promoter and that they are produced in approximately equivalent amounts, as judged by the yield obtained upon isolating the two peptides.

Most bacteriocins produced by lactic acid bacteria studied so far mediate their bactericidal activity through the formation of nonselective pores. Here we demonstrate that lactococcin G causes the selective efflux of potassium ions from sensitive cells. This phenomenon is unique, as it requires the combined action of the α and β peptides of lactococcin G. No efflux of potassium was observed in the absence of the complementary peptide, consistent with the fact that both peptides are required to obtain a bactericidal effect. The flux is highly selective, as cells retain the ability to maintain the ΔpH in the presence of lactococcin G, implying that protons are not released. As a consequence of the electrogenic potassium efflux, the Δψ collapses and the uptake of amino acids is inhibited. Cell death is finally effected by a futile cycle of ATP-driven potassium uptake and lactococcin G-mediated potassium release in combination with increased ATP hydrolysis by the F_0F_1-ATPase (Fig. 8). This results in a rapid depletion of the intracellular ATP pool, a cessation of ATP-dependent processes such as glutamate uptake, and an inability to maintain the osmotic balance.

Circulation of potassium ions is important for several homeostatic mechanisms, such as the regulation of intracellular pH and osmotic strength (12). Although little is known about the mechanism of potassium transport in *Lactococcus* species (2), the major uptake system appears to be an electrogenic potassium ATPase that is constitutively expressed. This system functionally resembles the Trk system of *Escherichia coli* and mediates the translocation of both potassium and rubidium ions (12). The electrogenic potassium uptake via this system is responsible for the rapid depolarization of the Δψ that is observed when lactococcal cells are supplied with a fermentable sugar (12). The ATP that is generated by the glycolytic pathway is used to accumulate potassium ions, which causes a depolarization of the Δψ. The subsequent action of the H^+-ATPase then results in a restoration of the Δψ and an increase of the intracellular pH and ΔpH. Lactococcin G thus interferes with one of the major homeostatic mechanisms of the cells, which may explain as to why it is so effective in its bactericidal action.

Other known two-component bacteriocins include the plantaricin A system (18), plantaricin S (reference 10 and unpublished results), lactacin F (1), and lactococcin M (27). Lactacin F also causes efflux of intracellular potassium, dissipation of the proton motive force, and hydrolysis of internal ATP in susceptible bacteria (1). Abee et al. (1) suspected that ATP hydrolysis, in the case of lactacin F, might be caused by efflux of P_i. This seems unlikely in our case since lactococcin G does not dissipate the ΔpH, indicating a highly specific pore. The mode of action of some one-component bacteriocins might have parallels with lactococcin G action: carnocin U149 (25) and lactostrepcin 5 (29) may act in a similar manner, as it has been shown that both bacteriocins cause hydrolysis of cellular ATP and the collapse of Δψ in target cells. Curiously, 20 mM potassium ions is able to protect the cells against lactostrepcin 5 and allowed them to maintain their ATP pool. This is not the case for lactococcin G (data not shown), and the precise mechanism of action under those conditions is unresolved.

Lactococcin G appears to be active against intact cells only, as all attempts to demonstrate an effect of this bacteriocin in membrane vesicles and liposomes were without success (data not shown). This may imply that other factors are required for a productive interaction with the cytoplasmic membrane, such as a cell wall component. Indications of involvement of a cell wall component have been obtained in the case of lactostrepcin that is produced by *Lactococcus* subsp. *cremoris* 202. Lactostrepcin 5 is inactive against protoplasts prepared from either sensitive or insensitive cells. Its activity is decreased about 10-fold after pretreatment of the cells with trypsin, suggesting the involvement of a proteinaceous factor at the cell surface (29). The involvement of such factors has also been suggested for lactococcin A (26), lactococcin B (28), and pediocin PA1 (4). Alternatively, it cannot be excluded that lactococcin G acts on a transport system which functions in intact cells but not in cell membrane vesicles and which is absent in liposomes. The observation that lactococcin G is still active on ATP-depleted cells indicates that an active ATP-consuming transport system is an unlikely target for the bacteriocin.

The study of the precise mechanism of potassium ion efflux and the role of the α and β peptides in this process awaits further detailed in vitro studies. The isolation and reconstitution of an eventual cell wall component are challenges for further research.

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